Tomography of Labeled Cryogenic Cell Nuclei using an X–Ray Microscope

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INTRODUCTION

The structure of proteins can be revealed by X-ray crystallography. With immunolabeling it is possible to localize these proteins in cells. Up to now light microscopy has mainly been used to study their distribution in cells by tagging the investigated protein with fluorophore-conjugated antibodies. While light microscopes allows routine investigation of whole, unsectioned cells, the obtainable resolution is diffraction limited to about 200 nm. In addition, this technique reveals mainly the distribution of the fluorophore-conjugated antibodies whereas most unlabeled cell structure is not clearly visualized. Electron microscopy can reveal cell structures at much higher resolution level, but is limited by the thickness of the sample, i.e. only up to 1 μm thick objects can be imaged. Therefore, no conventional imaging technique exists which can visualize the three-dimensional distribution of proteins inside whole hydrated cells, e.g. in the cell nucleus, with higher than light microscopical resolution. Due to the shorter wavelengths of X-rays than visible light, X-ray microscopy provides higher resolving power than light microscopes. By utilizing the natural absorption contrast between protein and water at photon energies of about 0.5 keV, smallest cell structures of about 30 nm size embedded in vitreous ice can be detected in X-ray microscope images [1, 2].

COMPUTED TOMOGRAPHY USING X-RAY MICROSCOPE IMAGES

Due to the short wavelengths of X-rays and low numerical aperture of the Fresnel zone plates used as X-ray objectives, the depth of field is several microns. Within the focal depth, imaging a thick specimen is to a good approximation equivalent to projecting the specimen absorption. Therefore, computed tomography based on a tilt series of X-ray microscopic images can be used to reconstruct the local linear absorption coefficient and image the three-dimensional specimen structure [3, 4]. To preserve the structural integrity of biological objects during image acquisition, microscopy is performed at cryogenic temperatures. This helps to stabilize the biological samples against radiation damage. At the resolution level of about 30 nm, it was demonstrated that biological cells in vitreous ice can tolerate a radiation dose about a 100 times higher than the dose limit observed in cryo TEM [1].

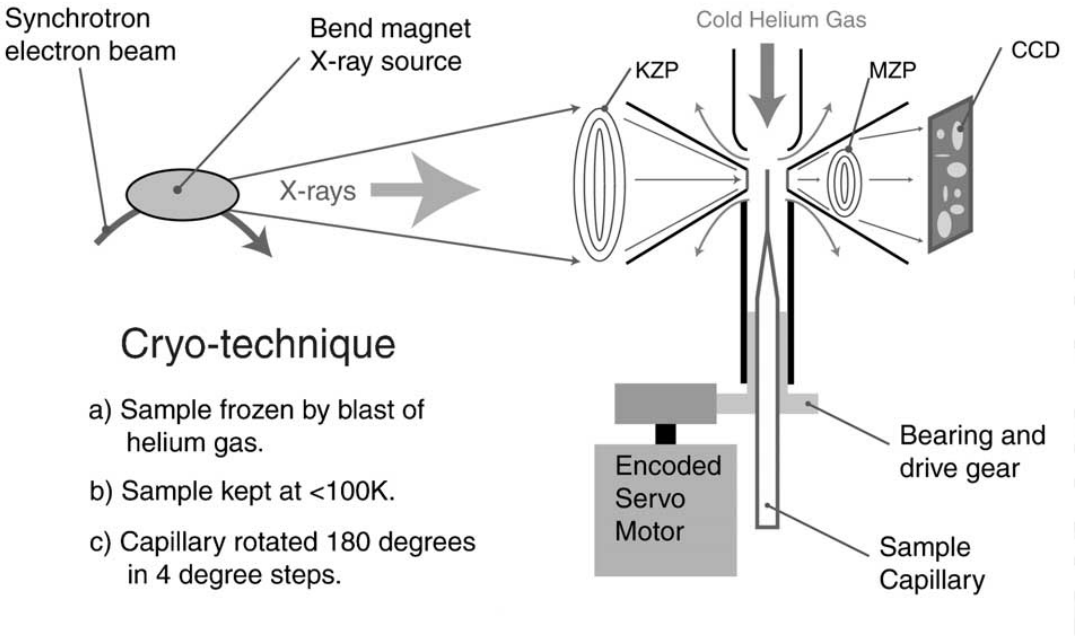

Figure 1: Schematic diagram of capillary based cryo tomography experiment performed using a soft X-ray transmission microscope, XM-1 at the ALS in Berkeley. A condenser zone plate lens (KZP) illuminates the sample with soft X-rays from a bend magnet X-ray source with 520 eV photon energy. An objective zone plate (MZP) generates a 2000× magnified image onto a back-thinned CCD camera. With the appropriate choice of illumination and MZP the depth of field of the XM-1 imaging system is close to 10 μm without significantly reduced lateral resolution. Under these conditions the magnified images are in good approximation projections of the specimens X-ray transmission. By rotating the capillary through 180 degrees in 4 degree angular steps, and recording the transmission image data at each orientation, the 3-D linear absorption coefficient has been reconstructed at about 60 nm resolution in all three spatial dimensions using multiplicative algebraic reconstruction techniques (MART).

Tomographic reconstructions of X-ray microscopic images have been used to compute the local three-dimensional (3D) linear absorption coefficient revealing the arrangement of internal structures of cells [5]. Combined with labeling techniques, nano-tomography is a new technique to study the 3D distribution of selected proteins inside whole cells.

Ideally tomography requires that an object is imaged under an angular range of 180 degrees. Therefore, a rotional symmetric sample holder has to be used. Thin glass capillaries with a wall thickness of about 250 nm fulfill this requirement and have sufficient transparency for soft X-rays with 0.5 keV photon energy. To avoid vibrations of such a thin sample holder in the X-ray microscope, the capillaries are electroplated with nickel, coating all but the final 400 microns which can be used for imaging samples.

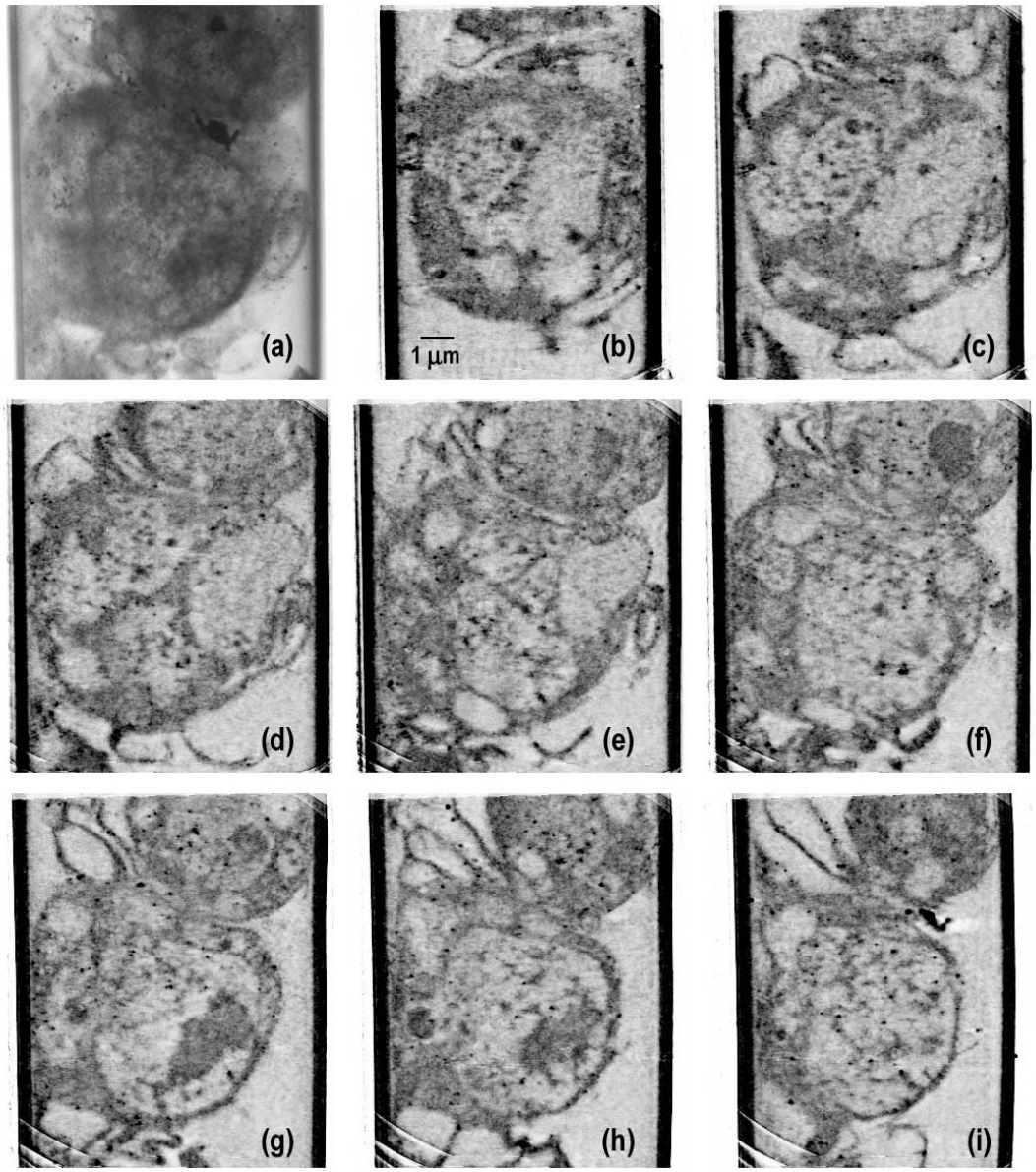

Figure 2: Panel (a) shows the first of the 48 X-ray microscope images of immunogold labeled *Drosophila melanogaster* cells constituting the tilt series, panels (b) – (h) show parallel slices of the reconstructed local linear absorption coefficient. The capillary axis is parallel to the plane of the paper. Each slice is 30 nm thick; they are located in a distance of 600 nm. Low absorption maps to light gray, and high absorption to dark gray. The distribution of the labeled nuclear protein inside the cell nuclei is visualized in the slices by the strong X-ray absorbing silver enhanced gold particles.

During aquisition of the tilt series, the specimen was held at a low temperature of about 110 K. The setup of the cryo transmission X-ray microscope (CTXM) at the ALS is shown in Fig. 1. In this CTXM liquid nitrogen cooled He gas is used to shock-freeze the sample in the capillary and to maintain it at low temperature.

In this work tomography based on X-ray microscopic images is applied to study the distribution of male specific lethal 1 (MSL-1), a nuclear protein involved in dosage compensation in *Drosphila melanogaster*. Dosage compensation ensures that males with single X chromosome have the same amount of most X-linked gene products as females with two X chromosomes (see e.g. [6].) To visualize the MSL-1 protein in the cell nuclei, the cells were labeled with a 1 nm colloidal gold conjugated antibody and then silver enhanced to increase the absorption contrast of the label. After filling the cell suspension into the capillaries, they were imaged at 0.52 keV photon energy with a zone plate objective with 40 nm outermost zone width. Fig. 2 shows the first of the 48 different viewing angles spanning an angular range of 180 degree. This tomographic data set was collected in 3 hours. In order to align all the different viewing angles to a common rotation axis, we added 100 nm colloidal gold particles as fiducial markers. The alignment can be done very precisely with about 10 nm accuracy, because only the center of the gold particles has to be determined. After alignment, a multiplicative algebraic reconstruction technique (MART) was applied to reconstruct the three-dimensional structure of the cells inside the capillary. Fig. 2 shows different 30 nm thick slices perpendicular through the capillary axis. The internal structure of the cells as well as the label and the added gold particles are shown with a resolution of about 60 nm.

In the future, we want to improve this technique with respect to resolution and specimen preparation. The resolution in the reconstruction can be significantly improved by reducing the angular step size from 4 degree to 1 degree to collect more viewing angles. In addition, fast-freezing with liquid ethane instead of cryogenic He gas will be applied to improve the vitrification of the hydrated samples. Finally, we plan to apply cryo X-ray nano-tomography in order to study different types of cells and their nuclear protein distributions.

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